



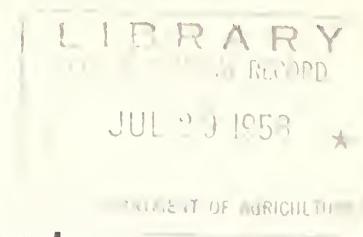
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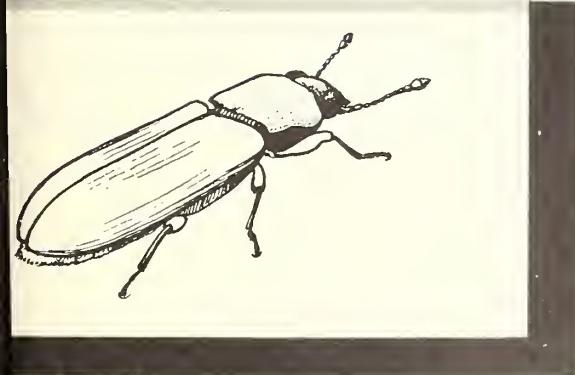


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Marketing Research Report No. 245



Evaluation of Ryania for the Protection of Stored Wheat and Shelled Corn from Insect Attack



Marketing Research Division
Agricultural Marketing Service
U. S. DEPARTMENT OF AGRICULTURE

WARNING

No tolerances have been established for the use of ryania as an insecticidal treatment for the prevention of insect infestation in stored grain. The tests reported herein were exploratory studies to develop information that could be used in considering the establishment of tolerances. Until such a tolerance is announced ryania protective treatments should not be used.

This report is the third of a group presenting results of tests with various insecticidal dusts and sprays applied to stored grain for protection against insect attack. These reports are a part of a broad program of research to reduce the cost of marketing farm products, including the cost of preventing insect infestation in stored grain.

July 1958

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ACKNOWLEDGMENTS

This report is based on research conducted by a number of individuals and groups. The performance tests were conducted by A. C. Apt, J. K. Quinlan, J. H. Schesser, G. H. Spitler, G. D. White, W. K. Whitney, and H. E. Fearing, of the Stored-Grain Insects Laboratory, Manhattan, Kans.

Determination of residues of ryania on treated wheat and in flour milled from treated wheat were made by S. B. Penick and Company. This company also supplied the ryania formulations.

The milling tests were made in cooperation with the Kelly Milling Company, Hutchinson, Kans. The commercial grade of grain used in the tests was determined by the Board of Grain Supervisors, Chicago, Ill.

SUMMARY

This report is the third of a series presenting results of tests with various insecticidal dusts and sprays applied to stored grain for protection from insect attack. It summarizes the tests with ryania begun in September 1952 and concluded in January 1956.

The studies were conducted under actual storage conditions both on the farm and at CCC bin sites. At the latter tests were made with wheat and shelled corn stored in the standard circular 3,250-bushel metal bins of the Commodity Credit Corporation, U. S. Department of Agriculture, and also on shelled corn in 3,000-bushel rectangular wooden bins.

After the dusts were applied, the grain was sampled monthly to determine the insect population trends and the moisture content of the grain. These samples were used to estimate the levels of residue, to observe any change in the potency of the residues in bio-assay tests, and to establish commercial grades. In tests with wheat, the level of residue was also traced through the milling process.

Application rates of ryania ranged from 20 to 100 pounds per 1,000 bushels in wheat, and from 20 to 60 pounds in corn. These rates practically eliminated any initial insect populations, and kept the grain almost insect-free for as long as 33 months.

Milling tests showed that 80 to 90 percent of the ryania could be removed in the scouring process preparatory to milling, and that relatively small amounts were carried into the feed and flour fractions.

There was little change in the overall moisture content of the grain during the storage period, and there was no apparent effect on the behavior of the protective dusts attributable to variations in moisture content. There were no changes in the commercial grade of the wheat during the period of test. A large portion of the corn was downgraded because of damage from moisture accumulation in the surface layer during the cooler months of the year. One series treated with ryania-sulfoxide formulation was downgraded because of odor.

In wheat, 9 species of stored-grain insects were found, the most abundant being the dermestids, the lesser grain borer, the flat grain beetle, and the saw-toothed grain beetle. In corn, 16 species were noted, the most abundant being the red flour beetle, the flat grain beetle, the saw-toothed grain beetle, and the Angoumois grain moth.

EVALUATION OF RYANIA FOR THE PROTECTION OF STORED WHEAT AND SHELLLED CORN FROM INSECT ATTACK

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BACKGROUND AND PURPOSE OF THE WORK

Grain handling and grain storage practices have changed during the past few years, and have resulted in the storage of grain for much longer periods of time, sometimes as long as 5 years. Reserve stocks of grain, particularly corn and wheat, have accumulated as a result of increased yields and the price support program. The safe storage of these reserve stocks has necessitated new and improved methods of preventing damage by insect attack. Emphasis is now being placed on preventive rather than curative measures of insect control.

In line with this trend, intensive studies were begun in the fall of 1952 to explore the possibility of applying insecticides directly to stored wheat and shelled corn to prevent the development of insect infestations. The use of insecticides for this purpose poses numerous problems. The fate of the resulting insecticidal residues must be ascertained to determine whether they will be within safe limits for the ultimate consumers of grain products--both human and livestock--and whether the grade of the grain is affected by the treatments.

This report is the third of a series presenting the results of the tests with various protective treatments. The data presented herein pertain to the studies in which ryania dusts were used. The data cover several phases of this complex problem; for presentation they are divided into five groups. The tests with both wheat and shelled corn are tabulated below:

Group I. Exploratory tests with farm-stored seed wheat in Reno County, Kans., begun in June 1952.

Group II. Tests with CCC-owned wheat at Beloit and LaCrosse, Kans., begun in June 1953.

Group III. Tests with CCC-owned shelled corn at Beattie, Kans., begun in September 1952.

Group IV. Tests with CCC-owned shelled corn in Illinois, begun in May 1953.

Group V. Tests with CCC-owned shelled corn in Saline Co., Mo., begun in July 1953.

MATERIALS

Three formulations of ryania were used in these tests. All were in dust form but no diluent was required since the ground ryania contains approximately 0.25 percent of ryanodine, the active agent and has good dusting properties.

¹ This laboratory is a field station of the Stored-Product Insects Section, Biological Sciences Branch, Marketing Research Division, Agricultural Marketing Service, U. S. Department of Agriculture.

1. Ground ryania wood or stems (95 percent passes through a 200-mesh screen).....	100.0 percent
2. Ground ryania	95.8 percent
Sulfoxide.....	3.2 percent
Inert	1.0 percent
3. Ground ryania	95.8 percent
N-Propyl isome	3.2 percent
Inert	1.0 percent

Most of the information in the following paragraphs was taken from an unnumbered publication of S. B. Penick & Co., 50 Church Street, New York 8, N. Y., dated 1953 and entitled "Ryania, a botanical insecticide."

Ryania is a tropical American genus of shrubs and small trees belonging to the family Flacourtiaceae, the principal source of the insecticide being the stem wood of Ryania speciosa Vahl, a species native to Trinidad. Other species occur in different areas of northern South America and in the Amazon basin.

Ryania was discovered in the course of a cooperative research program by the Research Laboratories of Merck and Co., Rahway, N. J., and the Department of Entomology of Rutgers University and the New Jersey Agricultural Experiment Station. This program was directed toward the survey of materials of botanical origin from all parts of the world in search of new materials of insecticidal value.

The first examination of Ryania under field conditions was made at the New Jersey Agricultural Experiment Station in 1943 using the ground root of Ryania speciosa at which time it was found to be outstandingly effective against the European corn borer.

An extensive investigation of the chemistry of Ryania principles has been conducted in Research Laboratories of Merck and Company. The active principles can be extracted with water and many organic solvents and quantitative extraction may be secured by the use of water, methanol, or chloroform. The active principle of Ryania is an alkaloid which has been designated as "Ryanodine".

Ryanodine is neutral to litmus and forms no salts. It is soluble in water, alcohol, acetone, ether, and chloroform; difficultly soluble in benzene; and insoluble in petroleum ether. The distribution coefficient of Ryanodine for ether/water is 1.3. The ultra-violet absorption spectrum shows a maximum at 2685 A° ($E:\% \text{ cm.} = 352$) in alcohol solution. A tentative empirical formula of $C_{25}H_{35}NO_9$ or $C_{26}H_{37}NO_9$ has been suggested for Ryanodine. The structural formula has not been established. Ryanodine has approximately 700 times the insecticidal potency of the stem wood of Ryania speciosa, and 0.25% of Ryania is considered to be Ryanodine.

Work with the physiological effects of Ryania indicate that: Ryanodine exerts a highly selective action on muscle tissue. It has been postulated that "Ryanodine acts by interfering with the high energy phosphate system in striated muscle." The characteristic action of Ryania is such as to produce a rapid cessation of normal activity, often without producing death for several days. "Insects in the state of 'flaccid paralysis' would be incapable of normal activity but could not be classified as dead."

To establish the safety of use of powdered Ryania stems as an insecticide, an extensive investigation was undertaken by the Merck Institute for Therapeutic Research.

This work has been reported by Heal and Kuna (1948)². They established the acute oral toxicity of powdered stem of Ryania speciosa to be 1200 mg./kg. in rats, 150 mg./kg. in dogs, more than 400 mg./kg. in monkeys, 650 mg./kg. in rabbits, 650 mg./kg. in mice, 2500 mg./kg. in guinea pigs, and more than 3,000 mg./kg. in chickens. Chronic oral toxicity studies with this same material demonstrated that it was possible to maintain rats, chickens or guinea pigs for at least 5 months on a diet containing 1 percent of the Ryania powder without producing symptoms of cumulative poisoning. Direct comparisons of this Ryania powder with DDT and cubé powders in chronic administration showed that Ryania powder is tolerated in larger amounts and for a longer period of time than either DDT or cubé.

TECHNIQUES

The tests were conducted in three types of bins: (1) Farm bins of wood construction with capacities ranging from 200 to 1,000 bushels; (2) USDA circular metal bins, 16 feet in height and 18 feet in diameter with a rated capacity of 3,250 bushels; (3) CCC rectangular wooden bins, 16 feet wide, 24 feet long, and 10 feet to the eaves with a rated capacity of 3,000 bushels.

The farm-stored wheat was dusted by estimating the number of bushels per truck load and sifting the required dosage of ryania uniformly over the surface of the load. The truck was unloaded into an auger hopper and the grain elevated into the bin. This method of handling was expected to produce a uniform distribution of the insecticide on the wheat. These treatments were applied at harvest in late June 1952.

In the CCC metal bins the dust was applied by hand as the grain ran from the auger, or by a mechanical applicator attached to the auger tube, or by aliquots as each truck load was emptied into the hopper of the auger.

In the rectangular wooden bins the applications were made by a mechanical applicator attached to the auger tube.

Sampling Methods

All of the sampling was done with an 11-celled grain trier equipped with extension handles. Samples were drawn monthly for determination of insect populations and moisture content of the grain.

In the farm bins, where wheat did not exceed 5 feet in depth, the samples were taken vertically from the center and near the wall, and horizontally from the top 2 to 3 inches in the center.

In the circular metal bins, the samples were taken vertically in the center of the bins from the top, middle, and bottom 5 feet, from the top 5 feet in each quadrant at a point about 3 feet from the wall, and horizontally from the top 2 to 3 inches at the center.

In the rectangular wooden bins, the samples were taken vertically from the top and bottom 5 feet in the center and at a point about 3 feet from the gable ends of the bins.

Statistical Analyses

The treated lots of grain in the CCC bins were compared with the untreated controls and with each other on the basis of the number of "bin months" of protection. The number of bin months of protection was computed by dividing the total number of months of pro-

² Heal, Ralph, E., and Kuna, Samuel. Toxicological and pharmacological studies on the powdered stem of Ryania speciosa, a plant insecticide. Jour. Pharmacol. and Expt. Therapeutics 93(4): 407-413. 1948.

tection for a series of bins having the same treatment by the number of bins in that series. The treatment was considered to give protection until the insect population reached the level at which it would be designated as "weevily" under the provisions of the U.S. Grain Standards current at the time of the tests. The grain was designated as "weevily" if a 1,000-gram sample contained living stored-grain insects as follows: Wheat: 2 or more weevils; or 1 weevil and 3 or more bran beetles; or 5 or more bran beetles. Corn: 2 or more weevils; or 1 weevil and 5 or more bran beetles; or 25 or more bran beetles. In order to classify the insects for grading purposes, the rice weevil, granary weevil, lesser grain borer, and the Angoumois grain moth were designated as weevils, and all other stored-grain insects as bran beetles.

The data thus obtained were tested statistically for reliability by the analysis of variance method. This method of statistical analysis establishes the significance of mean differences between treated lots or between treated lots and the untreated controls.

Residue Determinations

As no chemical method for the determination of ryania residues in wheat are known, the residues were estimated by exposing mosquito larvae to extractions from samples of treated grain. This method is not as satisfactory as a chemical one would be, but it does indicate the degree of ryania residue present.

Bioassay Tests

Bioassay tests were conducted for the purpose of observing any changes in the potency of the ryania during the storage period after treatment. In these tests 25 adults of rice weevils or of confused flour beetles were confined in 3 ounces of corn or wheat from both the treated and the untreated bins and the resulting mortalities were recorded after 1 and 3 weeks.

TESTS WITH WHEAT

Group I: Exploratory Tests with Farm-Stored Seed Wheat in Reno County, Kans., begun in 1952

All of the wheat used in these tests was of good, dry storage quality, the moisture content ranging from 11.0 to 12.0 percent. A total of 10 farm bins were treated with ryania dust: 2 at a dosage rate of 60 pounds per 1,000 bushels of wheat, 5 at 75 pounds, and 3 at 100 pounds.

Insect Population Trends

The monthly samples were taken to the laboratory and screened, and the number and species of stored-grain insects were recorded. In all of the 10 bins in which wheat remained in storage for from 6 to 20 months, only 2 insects--1 flat grain beetle and 1 Indian-meal moth--were found during the entire period. These two insects were in a bin receiving the 60-pound dosage rate. The results of these tests demonstrate that ryania gave almost complete protection against insects over a period of 20 months, and could be presumed to protect for an indefinite period. The reason for the variable lengths of storage periods was that the wheat could be removed at the option of the cooperating farmer.

Insecticidal Residues

The first samples for estimating the residue were drawn in July 1952, a month after the ryania was applied. The results are given in table 1. The estimated level ranged from 54 percent from the 75-pound dosage rate to about 70 percent from the 60- and 100-pound

dosage rates. This great variability may have been due in part to uneven distribution of the insecticide, or to sampling error.

TABLE 1.--Residues of ryania in farm-stored wheat 1 month after treatment; determined by the bioassay method

Application rate and test number	Ryania	Ryanodine ¹
60 lb./1,000 bu.		
1.....	P.p.m. 730	P.p.m. 1.8
2.....	690	1.7
Mean.....	710	1.8
75 lb./1,000 bu.		
3.....	1,230	3.1
4.....	180	.5
5.....	240	.6
6.....	110	.3
7.....	1,600	4.0
Mean.....	672	1.7
100 lb./1,000 bu.		
8.....	920	2.3
9.....	1,000	2.5
10.....	1,560	3.9
Mean.....	1,160	2.9

¹ Calculated on the basis of an estimated average of 0.25 percent of ryanodine in the pulverized ryania.

In April 1953, two 1-gallon samples were drawn from one bin in the farm-stored series that had received the 60-pound dosage rate. One sample was cleaned and scoured by the premilling process, and the other was left as it came from the bin. Table 2 shows that 31 percent of the amount of ryania applied was recovered in the unscoured sample, and of that amount 79 percent was removed by the cleaning and scouring process. These samples were not milled.

TABLE 2.--Residues of ryania in wheat before and after cleaning and scouring preparatory to milling; dosage rate 60 pounds per 1,000 bushels

Sample treatment	Ryania recovered	Ryanodine recovered
Unscoured, as wheat came from bin: ¹		
First portion.....	P.p.m. 300	P.p.m. 0.75
Second portion.....	300	.75
Third portion.....	320	.80
Mean.....	307	.77
Scoured, single sample.....	64	.16

¹ The unscoured sample was divided into 3 portions for bioassay tests.

A third set of samples, taken in June 1953, showed that approximately 90 percent of the ryania had been removed in the cleaning and scouring process. These samples were not milled.

In January 1954 a fourth set of samples were taken from 2 bins of wheat that had received the 75-pound dosage rate. The check sample was taken from a bin of untreated wheat of the 1953 crop. These samples were milled with an Allis-Chalmers experimental flour mill, and residues estimated for uncleared wheat, scoured wheat, milled feed (bran, shorts, and germ), and flour. Virtually all of the ryania was removed in the scouring process; less than 1 percent of the original application was carried through the milling process into the feed and flour fractions (table 3).

TABLE 3.--Residue in various milling products from wheat treated with ryania dust at the rate of 75 pounds per 1,000 bushels; treated June 1952 and milled January 1954

Milling fraction test number and moisture	Proportion of original weight of wheat	Amount of ryania found
	Percent 100	P.p.m.
Wheat as drawn from bin:		
11 (moisture 11.5%).....		¹ 195
12 (moisture 11.3%).....		² 2,625
Mean.....		1,410
Wheat after cleaning and scouring:	(³)	
11.....		¹ 24
12.....		¹ 46
Mean.....		15
Bran, shorts, and germ:		
11.....	29.2	⁴ 12
12.....	30.3	⁴ 12
Mean.....		⁴ 12
Flour:		
11.....	70.8	⁴ 14
12.....	69.7	⁴ 9
Mean.....		⁴ 12

¹ Mean of 2 tests.

² Mean of 4 tests. The great excess over the amount applied may have been due to agglomeration of the ryania as the bin was filled.

³ Not determined.

⁴ These values are not significant since they merely indicate the lower limit of the assay method for the size of samples used in the bioassays.

Changes in the Moisture Content During Storage

There was virtually no change in the moisture content of the wheat during the storage period, nor was there evidence that the protective treatment had any effect on the transfer of moisture or the amount of moisture.

Changes in the Commercial Grade

To obtain information on the possible downgrading of wheat treated with ryania dust, three 1,000-gram samples were treated at dosage rates of 60, 75, and 100 pounds per 1,000 bushels. These samples were submitted to the Chicago Board of Grain Supervisors for an opinion as to the effect of the treatments on the commercial grade of the wheat, particularly on the feel and odor. The opinion of the Board is quoted below:

The appearance is very likely not affected because no change is noted in the appearance of the three samples submitted. We do detect something of an odor and a

feeling which we recognize as not entirely natural. This odor and feel is more noticeable progressively with the samples containing the larger amounts of insecticide. We believe that the presence of the insecticide is more noticeable in the feel of the wheat than it is by odor, though immediately after smelling the samples some of us experienced a slight burning of the nose. Because of this it is possible that wheat treated with larger amounts of the insecticide may in some instances be not acceptable on a straight numerical grade.

Two months after treatment (August 1952) samples were drawn from each of the 10 treated bins and submitted to the Chicago Board of Grain Supervisors for an opinion as to the feel and odor of the wheat. The opinion of the Board is quoted below:

The 10 samples treated with ryania dust at a dosage of 60, 75, and 100 pounds per 1,000 bushels of wheat, are very dry and in the present state do not have an objectionable feel although you can detect evidence of treatment from the gritty-like feel of your hands. This gritty-like feel remains until your hands are washed. The samples treated at the rates of 60 and 100 pounds feel better than the samples treated with 75 pounds. [Note: Unknown to the inspector the 75-pound samples and the 100-pound samples had been inadvertently reversed in numbering. This would account for the difference noted by the inspector.] All samples have an odor not natural to wheat. We will not say it is objectionable but again we find that it burns our nostrils, and when our lips are moist we experience a burning sensation after smelling the samples.

Subsequently a number of samples taken from the treated bins were submitted for commercial grade and all were acceptable as to feel and odor.

Group II: Tests with CCC-Owned Wheat at Beloit and LaCrosse, Kans., Begun in 1953

The wheat used in these tests was of the 1952 crop, delivered from farm storage and placed in CCC storage in the spring of 1953.

These studies were directed toward determining the degree of protection afforded by the different dosage levels, and the length of time that the ryania retained its potency under the conditions imposed by metal bin storage. The degree of protection was determined by observation of the insect population trends, and the rate of degradation shown by the bioassay tests.

The ryania dusts were applied in June 1953 to wheat at the Beloit and LaCrosse bin sites. Six bins were treated at the rate of 20 pounds per 1,000 bushels, 6 bins at 30 pounds, and 6 at 50 pounds. Six untreated bins served as checks. The ryania was applied in aliquots to each truck load as the wheat was unloaded from the trucks into the auger hopper. Each bin was sampled monthly from June 1953 to January 1956 to determine insect population trends and the moisture content of the wheat. Initial and terminal samples were taken for commercial grades.

Insect Population Trends

The insect populations in the treated and check bins are presented in table 4. All three levels of treatment gave excellent protection from June 1953 to October 1954.

At the 20-pound dosage rate, the insect population in one bin (test 16) increased to the weevily level in October 1954; another in March 1955 (test 14); another in October 1955 (test 18); the three remaining bins carried through until the tests were terminated in January 1956.

At the 30-pound dosage rate, the insect populations were kept below the weevily level throughout the entire storage period.

TABLE 4.--Insect populations in wheat following applications of ryania dust at specified rates, June 1953

Application rate of ryania and test number	Living insects found per 1,000 grams of wheat in--																	
	1953								1954									
	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.		
20 lb./1,000 bu. 13.....	No. 0	No. 0	No. 0	No. 0	No. 0	No. 2.0	No. .8	No. 0	No. 0	No. 0	No. 0	No. 0	No. 0	No. .8	No. .3	No. 0	No. 0	No. 0
14.....	0	0	0	.3	0	.5	0	0	0	0	0	0	0	0	0	0	0	0
15.....	0	0	0	0	.3	0	0	0	0	0	0	0	0	0	0	0	0	0
16.....	0	1.0	.3	1.0	0	0	-	.3	0	0	0	0	0	0	0	0	.3	0
17.....	0	0	0	.3	0	0	-	.3	0	.5	0	0	0	0	0	0	0	0
18.....	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
30 lb./1,000 bu. 19.....	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
20.....	0	0	.3	.3	0	0	.8	0	0	0	0	0	0	0	0	0	0	.3
21.....	0	.3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
22.....	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
23.....	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.3
24.....	.5	0	0	.5	0	0	-	.8	0	.3	0	0	0	0	0	0	0	0
50 lb./1,000 bu. 25.....	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-
26.....	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
27.....	0	0	0	0	0	0	.3	0	0	0	0	0	0	0	0	0	0	0
28.....	0	1.0	.3	.3	.8	.8	-	0	0	0	0	0	0	0	0	0	0	0
29.....	.3	0	0	0	0	0	-	0	0	0	0	0	0	0	0	0	.3	0
30.....	0	0	0	0	0	0	-	0	0	0	0	0	0	0	0	0	.8	1.5
No treatment--controls 31.....	0	0	.8	2.0	314.5													
32.....	.3	0	2.0	1.0	1.5	.8	1.0	.5	.3	.3	0	0	0	.8	4.5	3.5	3.0	
33.....	1.0	4.3	1.5	2.8	2.0	1.0	35.8											
34.....	0	0	0	.3	.5	.3	-	0	.5	0	0	0	0	0	0	0	.3	1.5
35.....	.3	.5	0	1.3	.3	.3	-	36.3										
36.....	0	0	0	.5	.5	.5	-	2.3	36.5									

Application rate of ryania and test number	Living insects found per 1,000 grams of wheat in--																	
	1954--Continued				1955										1956			
	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.		
20 lb./1,000 bu. 13.....	No. 0	No. 0	No. 0	No. 0	No. 0	No. 36.0	No. 0	No. 0	No. 0	No. 0	No. 1.3	No. -	No. 2.5	No. .3	No. 0	No. -	No. 20	
14.....	.8	1.0	.5	0	0	.3												
15.....	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
16.....	.7	.8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
17.....	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
18.....	0	0	.5	0	0	.5	0	.3	.3	0	.5	-	38.5					
30 lb./1,000 bu. 19.....	.3	0	0	0	0	.3	0	0	0	0	.3	-	0	0	0	0	0	.23
20.....	0	0	0	0	0	0	0	0	0	0	0	0	.3	0	0	0	0	.23
21.....	0	0	0	0	0	0	.3	0	0	0	.5	-	2.3	3.8	-	20		
22.....	0	0	0	0	0	0	0	0	0	0	0	0	0	.5	-	20		
23.....	0	0	0	0	0	0	0	0	0	.3	0	0	.5	-	.3	-	.23	
24.....	0	1.5	0	0	.5	.5	.3	.3	0	.3	.5	-	.5	1.0	-	.23		
50 lb./1,000 bu. 25.....	0	0	0	0	0	0	0	0	0	0	.3	-	327.5	0	0	0	0	.20
26.....	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.5	2.0	-	.20
27.....	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.5	0	-	.23
28.....	0	.3	.3	.3	.3	2.3	1.0	.5	1.5	0	345.8	-	.8	2.0	-	.5	-	
29.....	0	.3	0	0	3.8													
30.....	4.0	39.0																
No treatment--controls 31.....																		
32.....	2.8	3.5	2.5	1.0	.5	3.0	0	.5	.3	1.5	310.3							
33.....	38.8																	

¹ No samples taken wherever dash appears.² Series terminated.³ Test terminated; grain reached weevily stage. After this count the bin was fumigated.

At the 50-pound dosage rate, the insect population reached the weevily level in one bin in November 1954 (test 30); another in August 1955 (test 29); another in October 1955; and the three remaining bins carried through until the series was terminated in January 1956.

All of the untreated controls became weevily before the end of the observation period.

Statistical Analyses of Performance

As stated previously the criterion for the elimination of a bin of wheat from a series was whether the grain would be designated as weevily according to U. S. grain standards. The mean number of months of protection for a series of bins was designated as the number of bin-months of protection. The bin-months of protection for the treated series compared with those of the controls are given below:

<u>Treatment</u>	<u>Bin-months of protection</u>
No treatment--controls.....	12.3
Rymania dust per 1,000 bushels of wheat:	
20 pounds	26.8
30 pounds	32.0
50 pounds	27.8

The differences between the treated lots and the untreated controls, or between treated lots, were tested for significance by the method of analysis of variance. This analysis showed that there were significant differences. The least significant differences between means were then computed. On the basis of this calculation there were found to be no significant differences between the means of the treated lots, but the differences between the treated lots and the untreated controls were significant.

This analyses showed that the period of protection was significantly longer in the treated series than that in the untreated controls. In a comparison of the treated lots, the analysis showed that there were no significant differences between the three dosage rates.

Bioassay Tests

Bioassay tests were conducted in order to observe any changes in the potency of the rymania during the storage period subsequent to treatment. In these tests 25 adults of rice weevils were confined in 3 ounces of wheat from both the treated and untreated bins, and the resultant mortality was recorded after 1 and 3 weeks. Table 5 shows that at the lowest dosage rate (20 pounds) there was a trend toward decreasing potency, but that at the two higher dosages the killing power remained about the same throughout the 15-month observation period.

Changes in the Moisture Content

The wheat used in these tests was dry, mostly below 11.0 percent moisture content. The moisture determinations of the samples drawn at monthly intervals during the observation period showed that there was a continual transfer of moisture within the grain mass. There was but little change in the overall moisture content, the usual moisture accumulation being noted in the surface grain during the cooler months of the year, followed by a redistribution of it during the warmer months. No effect of moisture on the protective treatment was observed in this series of tests.

Changes in the Commercial Grade

The commercial grades of the different bins of wheat, established at the beginning of the study, at quarterly intervals during the observation period, and at the termination of the observations in each bin, showed that there was no change in grade either because of treatment or changes in grade factors.

TESTS WITH SHELLLED CORN

These studies with shelled corn were directed toward the determination of the degree of protection against insect infestation provided by the rymania formulations, changes in

TABLE 5.--Mortality of adult rice weevils in bioassay tests with samples taken at intervals from wheat treated with ryania protective dust

Application rate and length of confinement	Mortality of weevils in samples taken in--					
	1953		1954			
	July	Oct.	Jan.	Apr.	July	Oct.
20 lb./1,000 bu.	Percent	Percent	Percent	Percent	Percent	Percent
1 week.....	100	40	70	15	4	22
3 weeks.....	100	100	79	74	59	87
30 lb./1,000 bu.						
1 week.....	100	63	76	17	39	28
3 weeks.....	100	100	100	96	99	96
50 lb./1,000 bu.						
1 week.....	100	72	83	16	25	24
3 weeks.....	100	100	100	95	96	98
No treatment--controls						
1 week.....	1	1	2	0	2	7
3 weeks.....	5	10	3	2	2	7

the moisture content, and changes in the commercial grades during the period of observation. The results of three groups of tests are presented herewith.

Group III: Tests With CCC-Owned Shelled Corn at Beattie, Kans., Begun in September 1952

The corn used in these tests was of the 1949 crop, and had been placed in storage in rectangular wooden bins in September 1950. The tests with ryania were begun in September 1952. Three formulations of ryania were used as stated in the section on materials. The dusts were applied with a mechanical applicator attached to the auger tube, as the corn was turned from one bin to another. Two bins were treated with the ryania dust at 45 pounds per 1,000 bushels and 2 at 60 pounds, 2 with ryania plus sulfoxide at 45 pounds and 1 at 60 pounds, and 2 with ryania plus *n*-propyl isome at 45 pounds and 1 at 60 pounds. After the bins were filled with the treated corn, a capping of dust was applied to the surface corn by running dust alone through the auger and depositing it on the surface corn. The corn was then leveled and raked, so that the insecticide was distributed throughout the top 6 inches of corn. Approximately 10 pounds of dust were used in capping each bin.

A series of three untreated bins served as controls, and these were not turned.

Insect Population Trends

The bins were sampled prior to treatment to establish the initial insect population density and distribution. After treatment the corn in each of the bins was sampled at monthly intervals and examined for insects. The results are given in table 6. The insect populations in the treated series were held at a low point throughout the observation period, whereas those in the control series increased to the weevily level by September 1953.

Statistical Analyses of Performance

The data on the performance of the ryania formulations were handled in the same manner as for wheat. Bins were dropped from a series if they became weevily, and the bin-months of protection were computed in the same manner as for wheat.

TABLE 6.--Insect populations in shelled corn following applications of ryania formulations at specified rates, September and December.

Formulation, application rate, and test number	Living insects found per 1,000 grams of shelled corn in--																								
	1952 ¹				1953												1954								
	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	
Ryania																									
45 lb./1,000 bu.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	
37.....	16.7	1.0	0.7	- ⁽²⁾	0	0	0	0.7	0	0.4	0	0.4	0	0	0	0	0	0	0	0	0	0	0	0	
38.....	53.3	3.7	2.3	-	3.0	.7	2.3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
60 lb./1,000 bu.																									
39.....	15.3	2.0	1.0	-	0	.4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
40.....	9.7	1.3	.4	-	.4	0	.4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Ryania-sulfoxide																									
45 lb./1,000 bu.																									
41.....	74.0	0	-	0	-	0	-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
42.....	2.2	2.7	-	.4	-	.4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
60 lb./1,000 bu.																									
43.....	53.5	0	-	0	-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Ryania-n-propyl isome																									
45 lb./1,000 bu.																									
44.....	2.3	0	-	0	-	0	-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
45.....	15.3	0	-	0	-	0	-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
60 lb./1,000 bu.																									
46.....	1.7	0	-	0	-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
No treatment--controls																									
47.....	3.0	3.0	4.0	4.7	5.1	0	1.4	1.4	1.4	0	2.7	9.0	2.7	9.0	620.0										
48.....	12.0	12.0	12.7	1.3	1.3	.4	.7	7.7	2.3	3.0	1.7	0	1.4	.4	630.0										
49.....	2.3	2.3	1.3	1.3																					
Formulation, application rate, and test number	Living insects found per 1,000 grams of shelled corn in--continued																								
	1954--continued												1955												1956
	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.					
Ryania	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	
45 lb./1,000 bu.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	
37.....	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
38.....	0	0	.7	1.0	0	0	.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
60 lb./1,000 bu.																									
39.....	0	0	0	0	.3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
40.....	0	0	0	0	.3	0	0	0	0	1.0	2.0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Ryania-sulfoxide																									
45 lb./1,000 bu.																									
41.....																									
42.....																									
60 lb./1,000 bu.																									
43.....																									
Ryania-n-propyl isome																									
45 lb./1,000 bu.																									
44.....	0	0	0	0	.3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
45.....	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
60 lb./1,000 bu.																									
46.....																									
No treatment--controls																									
47.....																									
48.....																									
49.....																									

¹ First samples taken prior to treatment.

² No samples taken wherever dash appears.

³ Series terminated.

⁴ Series terminated. Downgraded to sample grade because of commercially objectionable foreign odor due to treatment.

⁵ Terminated - excess moisture.

⁶ Insect population reached the "weevily" level. Bin dropped from series and fumigated.

The bin-months of protection for the Group III series of tests are tabulated below:

Treatment	Bin-months of protection
No treatment--controls.....	11
Ryania dust per 1,000 bushels of corn:	
45 pounds.....	40
60 pounds.....	40
Ryania-n-propyl isome per 1,000 bushels of corn:	
40 pounds.....	40

The bins treated with the ryania-sulfoxide formulation are not included in the tabulation because the sulfoxide imparted an objectionable odor to the corn. The records for the bin receiving the 60-pound dosage rate of the ryania-n-propyl isome formulation were omitted because the corn had to be disposed of due to excessively high moisture before the test was completed.

When tested for significance by the method of the analysis of variance, the mean difference between the treated series was not significant, but the difference between the treated lots and the untreated controls was highly significant. The variation ratio "F" was 1401.7, a much greater value than the 5.41 required for significance for this group.

The highly significant difference between the treated lots and the controls demonstrated that the dosage rates were greater than needed for effective protection over the 40-month observation period.

Bioassay tests

In the bioassay tests, the depletion of the ryania and the synergized formulations of it proceeded very slowly during the period of observation (table 7).

Changes in the Moisture Content

The corn used in these tests had been in storage for 2 years before the protective dusts were applied, and was dry enough for storage. The usual seasonal transfer of moisture within the grain mass was noted, but there was no overall change in the moisture content. There was no apparent effect on the performance of the protective dusts attributable to variations in moisture content.

Changes in the Commercial Grade

No change in commercial grade of the corn was noted except in that treated with the ryania-sulfoxide formulation. In the opinion of the Chicago Board of Grain Supervisors, this corn was not acceptable because of a rancid lard odor. Such corn was graded Sample COFO (commercially objectionable foreign odor). Corn treated with the other formulations was acceptable.

Group IV: Tests with CCC-Owned Shelled Corn in Illinois Begun in May 1953

The corn used in these tests was of the 1952 crop and in May 1953 was shelled on the farm and delivered to CCC bin sites in Illinois and stored in USDA 3,250-bushel metal bins. The dusts were applied with a mechanical applicator attached to the auger tube as the corn was unloaded from the delivery trucks.

One series of 10 bins was treated with ryania dust at a dosage rate of 20 pounds per 1,000 bushels, and another series of 11 bins at 30 pounds per 1,000 bushels. A third series of 19 bins receiving no treatment served as controls.

Insect Population Trends

After the bins were filled, samples were drawn at monthly intervals in the manner described for circular metal bins (p. 3). The samples were taken to the laboratory and the number of insects and the moisture content determined. The results are given in table 8. Even though much of the corn was of high moisture content, and thus provided a favorable environment for insect population increase, none of the treated series became weevily. Six of the 19 bins in the untreated control series became weevily within 4 months after they were filled, and 5 others developed insect populations exceeding 10 per 1,000 grams of corn. The entire series had to be terminated in November 1953 as much of the corn stored on these sites became unmanageable because of excessively high moisture content.

TABLE 7.--Mortality of adult rice weevils and confused flour beetles in bioassay tests with shelled corn treated with various ryania formulations at specified rates.

Application rate and length of confinement	Confused flour beetles						Rice weevils						1955	
	1952			1953			1954			1954				
	Oct.	Nov.	Jan.	Apr.	June	Aug.	Dec.	Mar.	June	Sept.	Dec.	Mar.		
Ryania	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	
60 lb./1,000 bu.	52	52	97	11	70	18	40	38	54	41	36	54	41	
1 week.....	99	6	86	58	50	68	86	74	70	86	74	
3 weeks.....	80	86	74	70	70	70	70	40	40	40	
45 lb./1,000 bu.	10	85	85	6	21	58	48	14	0	44	36	36	18	
1 week.....	100	76	92	74	80	8	70	70	40	40	54	
3 weeks.....	
Ryania-sulfoxide	8	77	77	82	19	68	80	96	96	96	96	96	96	
60 lb./1,000 bu.	44	44	10	11	21	9	86	70	100	100	100	100	100	
1 week.....	75	86	70	70	70	100	100	100	100	100	
3 weeks.....	
45 lb./1,000 bu.	10	96	96	11	32	100	100	100	100	100	100	100	100	
1 week.....	75	86	100	100	100	100	100	100	100	100	
3 weeks.....	
Ryania-n-propyl isome.	22	99	99	36	32	100	100	100	100	100	100	100	100	
60 lb./1,000 bu.	64	64	11	21	96	92	92	92	92	92	92	92	92	
1 week.....	75	86	100	100	100	100	100	100	100	100	100	
3 weeks.....	
45 lb./1,000 bu.	14	97	11	21	96	92	92	92	92	92	92	92	92	
1 week.....	75	86	100	100	100	100	100	100	100	100	100	
3 weeks.....	
No treatment--controls	1	1	11	1	8	5	2	0	2	2	2	2	2	
1 week.....	17	6	3	2	2	2	2	2	2	2	
3 weeks.....	

TABLE 8.--Insect populations in shelled corn in Illinois following application of ryania dust at specified rates, May 1953

Dosage rate and test number	Living insects found per 1,000 grams of shelled corn in-					
	June	July	Aug.	Sept.	Oct.	Nov. ¹
20 lb./1,000 bu.						
50.....	Number	Number	Number	Number	Number	Number
50.....	0	0	0.3	0.8	0	0
51.....	1.3	.5	.3	.5	4.0	0.3
52.....	0	0	1.5	1.0		
53.....	0	0.3	0	0	.8	0.3
54.....	0	0	1.8			
55.....	0	0	1.0			
56.....	0	0	0	1.5		
57.....	0	.3	0	1.3		
58.....	.5	.3	1.0	0	1.0	
59.....	0	0	0	0	0	0
30 lb./1,000 bu.						
60.....	0	2.5	3.0	.8	0	0
61.....	0	0	0	.3	0	0
62.....	0	0	1.5	0	1.3	.8
63.....	0	0	0	0	1.0	
64.....	0	.3	0	0	1.0	
65.....	0	.8	1.0			
66.....	0	0	1.0			
67.....	0	0	.5	1.0		
68.....	.3	0	0	0		
69.....	0	0	1.0	0	1.0	
70.....	0	0	0	.3	.3	0.5
No treatment--controls						
71.....	.3	.3	3.5	112.3		
72.....	0	7.3	234.5			
73.....	0	3.3	239.0			
74.....	0.3	2.3	2.0	14.8		
75.....	0.5	2.0	4.8	2.5	14.0	20.3
76.....	0	11.5	7.5	-	15.0	12.3
77.....	1.0	8.3	20.0	-	17.5	16.5
78.....	0	1.0				
79.....	0	3.8	8.3	17.3	231.5	
80.....	0.5	0	1.0			
81.....	0	5.0	12.8			
82.....	0	.8	3.5	2.0	9.5	
83.....	0	5.5	1.0			
84.....	.3	3.0	2.5	15.5		
85.....	1.0	0	1.8			
86.....	.5	.5	1.8	249.3		
87.....	.1	4.3	22.3			
88.....	.5	1.8	1.5	16.8		
89.....	0	4.5	6.0	212.3		

¹ The Illinois series of bins had to be terminated in November 1953 as much of the corn stored on these sites became unmanageable because of high moisture content.

² Test terminated; grain reached weevily stage. After this count the bin was fumigated.

Statistical Analysis of Performance

The data on the performance of ryania in stored corn in Illinois (Group IV) were handled in the same manner as for Group III. The bin months of protection for the Group IV series are given below:

<u>Treatment</u>	<u>Bin-months of protection</u>
No treatment--controls.....	3.8
Ryania dust per 1,000 bushels of corn:	
20 pounds.....	4.7
30 pounds.. ..	4.8

When tested for significance by the method of the analysis of variance there were no significant differences between the means of either the treated bins or between the treated bins and the untreated controls. The variation ratio of 2.56 was less than the value of 3.25 required for significance for this group. This was due to the short time that the series were under observation.

Changes in Moisture Content

As noted previously, much of the corn put into storage on CCC bin sites in the spring of 1953 contained too much moisture to store safely. The moisture content of the corn ranged from 11.4 to 16.1 percent, with some loads running as high as 19.0 percent. For this reason much of it became unmanageable.

Changes in the Commercial Grade

The commercial grades of the bins of corn under observation taken at the beginning and end of the storage period showed that there was no downgrading due to the treatment. Of 18 bins in Group IV, 10 were graded sample on receipt at the bin site, and the other 8, received as number 1, 2, or 3 yellow, were downgraded to sample at the end of storage because of the increase in the amount of total damage.

Group V: Tests with CCC-Owned Shelled Corn in Saline County, Mo., begun in July 1953

The corn used was of the 1952 crop and was delivered to CCC bin sites in Saline County, Mo., during July 1953. The dusts were applied with a mechanical applicator attached to the tube of the loading auger as the corn was unloaded from the delivery trucks.

Three series of bins were treated with ryania dust at rates of 20, 30, or 50 pounds per 1,000 bushels of corn.

A fourth series of 7 bins receiving no treatment served as controls.

Insect Population Trends

After the treatments had been applied, samples were drawn at monthly intervals in the manner previously described for circular metal bins. The samples were taken to the laboratory and the number of insects and the moisture content determined. The results are given in table 9. In the treated series excessive moisture in the corn caused more trouble than insect infestation.

In the series treated with 20 pounds per 1,000 bushels, one bin of corn became weevily in March 1955, another in August 1955, one was dropped because of excessive moisture content, and the fourth was carried through until the test was terminated in February 1956.

TABLE 9---Insect populations in shelled corn following application of ryania dust at specified rates in Saline County, Mo., in August 1953

Application rate of ryania and test number	Living insects found per 1,000 grams of shelled corn in--																			
	1953					1954														
	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.			
20 lb./1,000 bu.	No.	No.	No.	No.	No.	No. ¹	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.
91.....	0	1.3	4.5	23.3	1.0	-	0	0	0	0	0.3	0	1.0	0.8	0	0.3	0	0	0	0
92.....	0	.5	4.3	8.0	5.3	-	.3	0	.3	2.3	.5	.3	2.0	0	.5	.8	0	0	0	0
93.....	0	.3	1.0	2.3	0	-	0	0	0	1.0	.5	0	0	0	0	0	0	0	0	0
94.....	0	2.3	4.5	1.0	0	-	0	0	0	3.3	3.3	0	0	.5	2.5	8.3	16.5			
30 lb./1,000 bu.																				
95.....	0	0	1.0	4.5	1.0	-	.3	40												
96.....	0	.5	5.5	29.5																
97.....	0	.5	2.8	213.0																
98.....	0	1.5	1.8	3.5	-	-	0	0	0	2.5	.5	0	.3	0	0	0	0	0	0	0
50 lb./1,000 bu.																				
99.....	.3	0	.3	.5	2.0	-	.3	0	.8	.5	.5	.3	.3	.3	.3	.3	.3	.3	0	0
100.....	.3	2.5	5.0	8.3	2.3	-	0	4.5												
101.....	0	1.3	0	1.3	.8	-	0	0	.3	0	0	0	0	0	0	0	0	0	0	0
102.....	-	1.0	.8	.8	0	-	40													
No treatment--controls																				
103.....	1.3	10.8	232.8																	
104.....	1.8	14.3	292.8																	
105.....	0	8.0	217.5																	
106.....	2.0	12.0	4.5	2.8	1.0	-	.3	0	.5	1.5	1.5	213.3								
107.....	0	8.3	237.5																	
108.....	.3	5.0	8.0	1.8	4.3	-	.5	0	.3	.3	.3	0	6.8	8.8	3.5	220.8				
109.....	.3	3.5	249.8																	

Application rate of ryania and test number	Living insects found per 1,000 grams of shelled corn in--																			
	1955							1956												
	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.						
20 lb./1,000 bu.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.						
91.....	0	0.3	0.3	1.3	0.3	0	0	22.3	1.8	1.5	0.8	-	2.3	-	31.0					
92.....	0	.5	1.0	0	0	0	1.8													
93.....	2.0	40																		
94.....	22.3	-	200.0																	
30 lb./1,000 bu.																				
95.....	0	0	0	0	0	0	0	0	0	0	.3	.3	0	-	-	-	-	3.3		
96.....																				
97.....																				
98.....	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
50 lb./1,000 bu.																				
99.....	0	0	0	0	0	0	0	1.0	1.0	2.3										
100.....	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
101.....	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
102.....	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
No treatment--controls																				
103.....																				
104.....																				
105.....																				
106.....																				
107.....																				
108.....																				
109.....																				

¹ No samples taken wherever dash appears.² Test terminated; grain reached weevily stage. After this count the bin was fumigated.³ Series terminated.⁴ Dropped from series because of excessive moisture.

In the series treated with 30 pounds, two bins became weevily in November 1953, one had to be dropped because of excessive moisture, and the fourth was carried through until the test was terminated in February 1956.

In the series treated with 50 pounds, the first weevily bin did not appear until October 1955, two had to be dropped because of excessive moisture, and the fourth was carried through until the test was terminated in February 1956.

In the untreated controls, the insect populations increased to the weevily level in 5 bins in October 1953, in 1 bin in July 1954, and in another in November 1954.

Statistical Analyses of Performance

The data on the performance of the formulations used in Group V were handled in the same manner as for the preceding groups. Bins were dropped from a series if they became

weevily and the number of bin-months of protection were computed. The bin-months of protection for the Group V series are tabulated below:

<u>Treatment</u>	<u>Bin-months of protection</u>
No treatment--controls.....	6.1
Rymania dust per 1,000 bushels of corn:	
50 pounds	35.0
30 pounds	12.3
20 pounds	24.7

The differences between the treated bins and the untreated controls, or between treated bins, were tested for significance by the method of the analysis of variance. This analysis showed that there were significant differences. The least significant differences between means were then computed. On the basis of this calculation, there were found to be significant differences between the untreated controls and the dosage rate of 50 pounds; the untreated controls and the dosage rate of 20 pounds; the 50 and the 30-pound dosage rates; and the 30 and the 20-pound dosage rates.

This analysis showed that the period of protection was significantly longer in the corn treated with 50 pounds and 20 pounds than in the untreated controls. The difference between the 30-pound rate and the controls was not significant due to the wide variability and small number of bins in the treated series that were not adversely affected by excessively high moisture content of the corn.

Changes in the Moisture Content

The corn used in the Group V tests was not sorted as to moisture content as it was delivered to the storage site. The result was that there were wide differences in the moisture contents of the different lots of corn put into the same bin. In average samples taken in August 1953 soon after filling the bins the moisture content ranged from 11.6 to 13.5 percent. Samples drawn in January 1956 showed that there had been but little change in moisture content during the storage period.

Changes in the Commercial Grade

No changes in the commercial grade were noted as being due to treatment. However, of 19 bins in the series, 15 of them were downgraded one or more grades because of increases in the grade factor "total damage". This was due largely to the accumulation of moisture in the surface corn which caused molding and caking.

COMPARATIVE ABUNDANCE OF THE SPECIES OF INSECTS

The monthly samples drawn from the bins were taken to the laboratory and the number and species of stored-grain insects were recorded. The comparative abundance of the different species in wheat and shelled corn is given in tables 10 and 11, expressed as percentages of the total number of insects found during 3-month periods.

In the treated wheat (table 10), 7 species of stored-grain insects were found, the most abundant species being the dermestids (Trogoderma spp.) followed by the lesser grain borer and the flat grain beetle. In the untreated controls, 9 species were recorded, the most abundant species being the lesser grain borer, followed by the dermestids and the saw-toothed grain beetle.

In the treated corn (table 11), 15 species of stored-grain insects were noted, the most abundant species being the red flour beetle, the Angoumois grain moth, and the foreign grain beetle, in the order named. In the untreated corn 16 species were found, the most abundant being the red flour beetle, the flat grain beetle, and the saw-toothed grain beetle.

TABLE 10.--Comparative abundance of the species of stored-grain insects found in the samples of wheat taken from the test bins, 1953-56

Source of samples and species of insects ¹	Proportion of species of insects in samples taken during--													
	1953			1953-54			1954-55			1955-56			Total insects	Percent of total
	June-July	Aug.-Oct.	Nov.-Jan.	Feb.-Apr.	May-July	Aug.-Oct.	Nov.-Jan.	Feb.-Apr.	May-July	Aug.-Oct.	Nov.-Jan.			
Treated Series:				Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Number	Percent	
Granary weevil.....	0	12.5	55.2	0	0	0	0	0	0	0	0	18	1.7	
Lesser grain borer.....	0	25.0	13.8	25.0	0	43.1	15.9	0	0	0	0	78	7.5	
Dermestidae ²	0	6.3	6.9	25.0	100.0	49.2	84.1	100.0	100.0	99.5	95.6	898	86.5	
Flat grain beetle ³	66.7	43.8	20.7	50.0	0	6.2	0	0	0	0	0	27	2.6	
Saw-toothed grain beetle	8.3	12.5	0	0	0	1.5	0	0	0	.5	2.9	8	.8	
Cadelle.....	25.0	0	0	0	0	0	0	0	0	0	0	3	.3	
Red flour beetle.....	0	0	3.4	0	0	0	0	0	0	0	1.5	2	.2	
Total number observed ⁴	(12)	(16)	(29)	(4)	(6)	(65)	(258)	(74)	(67)	(435)	(68)	(1034)		
Control series--														
No treatment														
Lesser grain borer.....	0	3.2	8.1	51.0	0	87.3	0	0	0	0	0	637	48.0	
Rice weevil.....	4.0	0	4.3	0	0	0	0	0	0	0	0	8	.6	
Granary weevil.....	0	5.6	1.2	0	0	0	0	0	0	0	0	9	.7	
Dermestidae ²	0	0	.6	0	8.4	.1	0	14.3	80.0	99.5	83.4	215	16.2	
Saw-toothed grain beetle	80.0	31.2	20.5	5.1	83.2	5.6	60.8	71.4	10.0	0	0	181	13.7	
Flat grain beetle ³	4.0	19.2	41.0	8.4	4.2	6.1	39.2	14.3	0	0	8.3	152	11.5	
Indian meal moth.....	0	34.4	10.0	0	4.2	0	0	10.0	.5	0	8.3	63	4.8	
Red flour beetle.....	8.0	6.4	11.8	35.5	0	.9	0	0	0	0	0	56	4.2	
Cadelle.....	4.0	0	2.5	0	0	0	0	0	0	0	0	5	.3	
Total number of insects observed ⁴ ...	(25)	(125)	(161)	(59)	(24)	(676)	(28)	(14)	(10)	(192)	(12)	(1326)		

¹ The scientific names of the insects listed herein can be found in Farmers' Bulletin 1260 (rev.) Aug. 1955.

² *Trogoderma* spp.

³ Probably a complex of 3 species: *Laemophloeus pusillus* (Schönh.) ; *L. ferrugineus* (Steph.) ; and *L. turcicus* Grouv.

⁴ Numbers in parentheses represent the total number of insects found during each period and are not percentages.

TABLE 11.--Comparative abundance of the species of stored-grain insects in the samples of shelled corn taken from the test bins, 1953-56

Source of samples and species of insects ¹	Proportion of species of insects in samples taken during--										Percent of total	
	1953		1953-54		1954-55		1955-56		Total insects			
	June-July	Aug.-Oct.	Nov.-Jan.	Feb.-Apr.	May-July	Aug.-Oct.	Nov.-Jan.	Feb.-Apr.	May-July	Aug.-Oct.	Nov.-Jan.	
Treated series:												
Rice weevil.....	9.7	12.2	1.1	0	0.8	2.0	5.9	1.0	87.5	22.0	29.2	87
Granary weevil.....	22.6	1.2	0	0	7.8	.5	0	0	0	26.0	0	28
Red flour beetle....	0	1.6	.7	0	0	19.6	82.2	0	0	0	0	0
Angoumois grain moth.....	6.5	65.7	52.2	10.0	95.2	49.0	2.3	0	0	4.0	0	998
Foreign grain beetle.....	0	13.8	33.6	10.0	0	0	0	0	0	0	0	556
Flat grain beetle ²	3.2	2.0	2.0	0	.8	13.7	5.9	.6	0	44.0	66.7	187
Larger black flour beetle.....	0	4.4	80.0	1.6	2.0	0	0	0	0	0	0	0
Indian-meal moth.....	41.9	.8	0	0	0	2.0	0.5	0	0	4.0	0	19
Two-banded fungus beetle.....	0	4.2	0	0	0	0	0	0	0	0	0	0
Saw-toothed grain beetle.....	16.1	.8	0	0	.8	0	2.7	0	0	0	0	14
Cadelle.....	0	1.2	.2	0	0	3.9	0	0	0	0	4.2	7
Hairy fungus beetle.....	0	.8	0	0	0	0	0	0	0	0	0	2
Red-horned grain beetle.....	0	0	0	0	.8	0	0	0	0	0	0	1
Yellow mealworm.....	0	0	0	0	0	0	0	0	12.5	0	0	1
Undetermined larvae.....	0	0	1.6	0	0	0	0	0	0	0	0	7
Total number of insects observed ³	(31)	(254)	(450)	(10)	(125)	(51)	(219)	(814)	(8)	(50)	(24)	(2036)
No treatment-controls:												
Rice weevil.....	7.2	.8	1.0	16.7	11.6	2.3	2.1	7.4	.3	5.0	85	1.8
Granary weevil.....	1.1	0	0	0	0	0	0	0	.1	0	4	.1
Red flour beetle....	0	34.8	65.4	0	4.3	28.7	65.0	34.0	0	4.6	25.5	1,494
Flat grain beetle ²	9.7	31.8	13.8	33.2	13.1	26.6	31.1	59.6	2.5	38.3	46.0	32.0
Saw-toothed grain beetle.....	25.8	13.5	2.8	16.7	65.2	14.5	0.2	0	82.6	54.7	6.2	27.3

See footnotes at end of table.

Continued

TABLE 11.--Comparative abundance of the species of stored-grain insects in the samples of shelled corn taken from the test bins, 1953-56--continued

Source of samples and species of insects ¹	Proportion of species of insects in samples taken during--												Percent of total
	1953			1953-54			1954-55			1955-56			
	June-July	Aug.-Oct.	Nov.-Jan.	Feb.-Apr.	May-July	Aug.-Oct.	Nov.-Jan.	Feb.-Apr.	May-July	Aug.-Oct.	Nov.-Jan.	Total Insects	
No treatment--													
controls (continued)	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Number	Percent
Indian-meal moth.....	15.8	4.5	0.1	16.7	0	24.8	1.0	4.2	1.1	9.9	2.52	5.4	
Foreign grain beetle...	11.8	6.5	11.3	0	0	0	0	0	0	0	0	207	4.4
Angoumois grain moth...	11.8	3.3	1.8	0	0	.4	0	0	0	0	0	90	1.9
Larger black flour beetle.....	2.3	2.3	1.6	16.7	0	.9	0	0	0	.3	1.2	51	1.1
Hairy fungus beetle...	13.6	2.3	.1	0	0	.6	0	0	0	.2	0	69	1.5
Dermestidae ⁴	0	0	0	0	0	.6	.2	0	0	.3	6.2	12	.3
Small-eyed flour beetle.....	0	0	1.6	0	0	0	0	0	0	0	0	15	.3
Cadelle.....	2.2	.2	.1	0	5.8	0	0	4.3	0	.1	0	17	.4
Corn sap beetle.....	0	0	0	0	0	.6	0	0	0	0	0	3	.1
Two-banded fungus beetle.....	0	0	0.4	0	0	0	0	0	0	0	0	4	.1
Red-horned grain beetle.....	.7	0	0	0	0	0	0	0	0	0	0	2	Trace ⁵
Total number of insects observed ³	(279)	(1066)	(923)	(6)	(69)	(516)	(428)	(47)	(121)	(1137)	(81)	(4673)	

¹ The scientific names of the insects listed herein can be found in Farmer's Bulletin 1260 rev. Aug. 1955.

² Probably a complex of 3 species: *Laemophloeus pusillus* (Schönh.) ; *L. ferrugineus* (Steph.) ; and *L. turcicus* Grouv.

³ Numbers in parentheses represent the total number of insects found during each period and are not percentages.

⁴ *Trogoderma* spp.

⁵ Trace = less than 0.1 percent.

FINDINGS

The following points were demonstrated in the tests reported herein:

1. Rymania formulations protected stored wheat and shelled corn through long periods of storage.
2. Dosage rates of 20 pounds per 1,000 bushels or higher in wheat and in shelled corn provided excellent protection from insect infestation through two full summer seasons after the year in which they were applied.
3. Bioassay tests showed that degradation of the rymania in the treated grain was very slow.
4. About 80 to 90 percent of the rymania could be removed from the treated grain by a simple sifting.
5. The residues in the milling fractions of wheat were about equally distributed in the feed and flour fractions and were reduced to relatively small amounts as related to the dosage rate.
6. The moisture content of the grain had no observable effect on the treatments.
7. The commercial grade of the grain was not affected except that of the shelled corn treated with the rymania-sulfoxide formulation, which imparted an objectionable odor.
8. Nine species of stored-grain insects were found in wheat and 16 species in shelled corn. The dominant species in wheat were the dermestids, the lesser grain borer, the flat grain beetle, and the saw-toothed grain beetle; in corn, the red flour beetle, the flat grain beetle, the saw-toothed grain beetle, and the Angoumois grain moth.

